

Figure S1

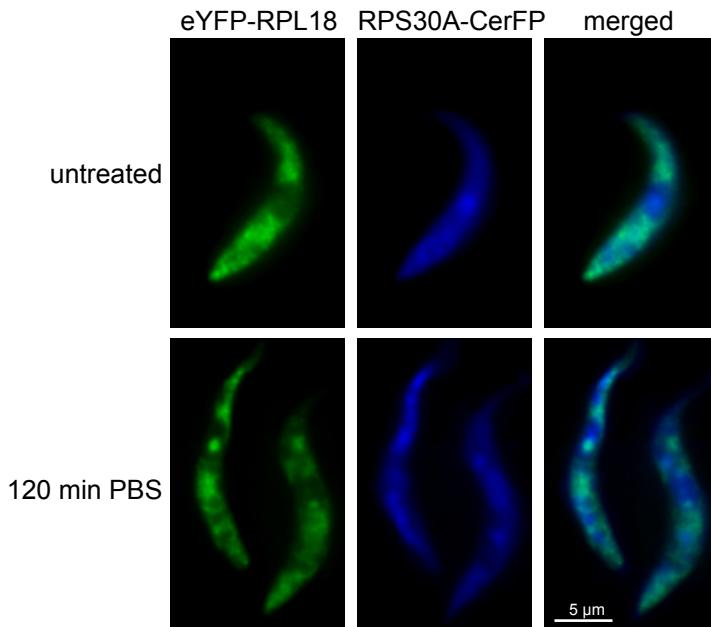


Figure S1. No evidence for the localization of trypanosome ribosomal subunits to starvation stress granules

Co-expression of an N-terminal eYFP fusion of *TbRPL18* and a C-terminal CerFP fusion of *TbRPS30A* from endogenous loci in procyclic trypanosomes. Fluorescence microscopy images (Z-stack projections) of untreated and starved (120 min PBS) cells are shown. An N-terminal fusion of the *Arabidopsis* RPL18 to a His₆-Flag tag integrates into polysomes (Zanetti et al., 2005) and a C-terminally GFP tagged RPS30 protein was used as a marker for the small ribosomal subunit in yeast, where it localizes to stress granules at robust heat shock (Grousl et al., 2009). Given that ribosomes are among the evolutionary most conserved structures, it is likely that the equivalent fusions of the trypanosome orthologues are functional.

Figure S2

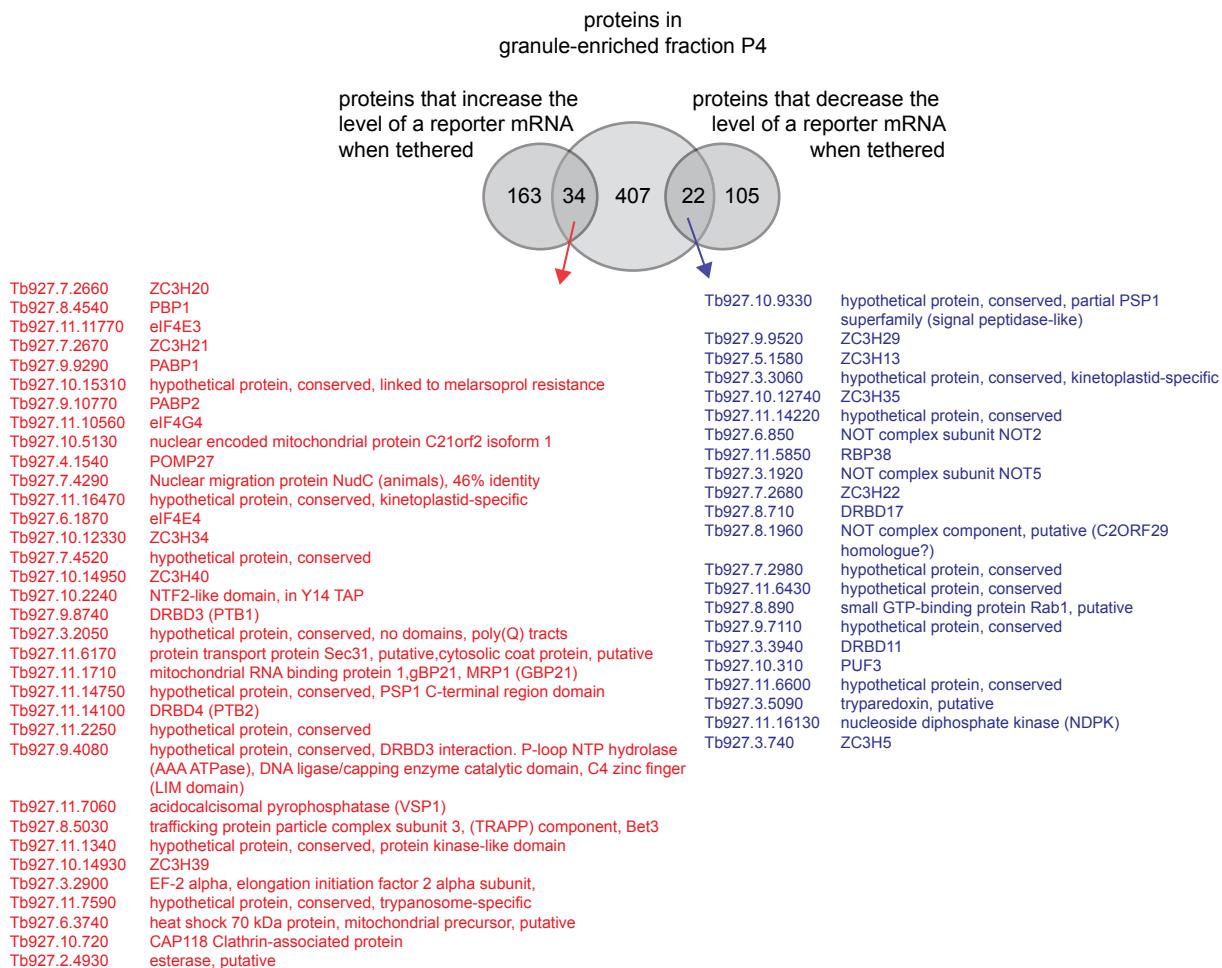


Figure S2. The 463 proteins of the granule-enriched fraction (P4) identified by mass spectrometry were compared with proteins identified as regulators of mRNA stability in a genome wide tethering screen (Erben et al (2014)). Proteins that were identified in both studies are shown in red (overlap with proteins that cause an increase in the level of the reporter mRNA) or blue (overlap with proteins that cause a decrease in the level of reporter mRNA).

Erben, E.D., Fadda, A., Lueong, S., Hoheisel, J.D. and Clayton, C.E. (2014) A genome-wide tethering screen reveals novel potential post-transcriptional regulators in *Trypanosoma brucei*. PLoS Pathog, 10, e1004178.

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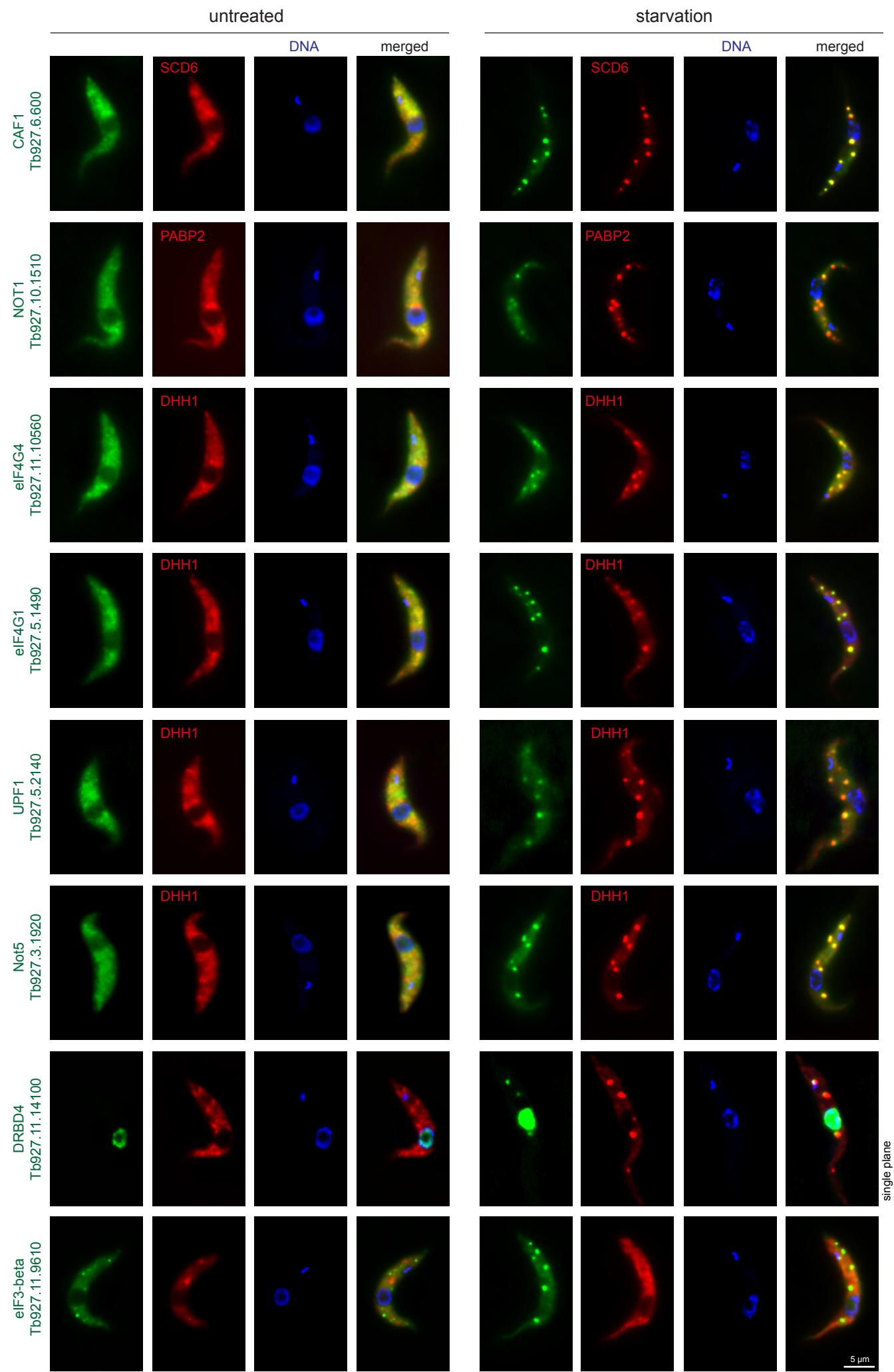


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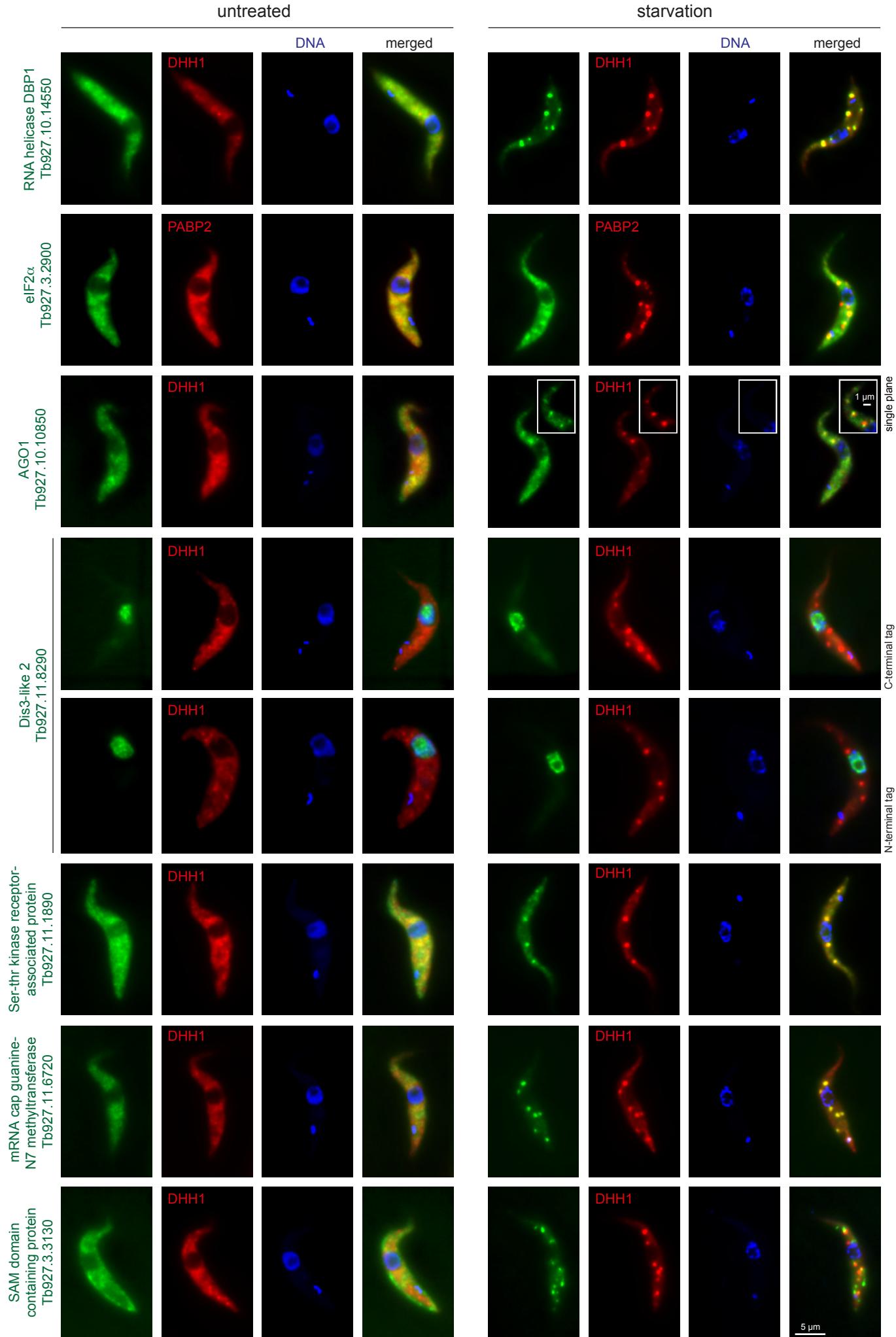


Figure S4A

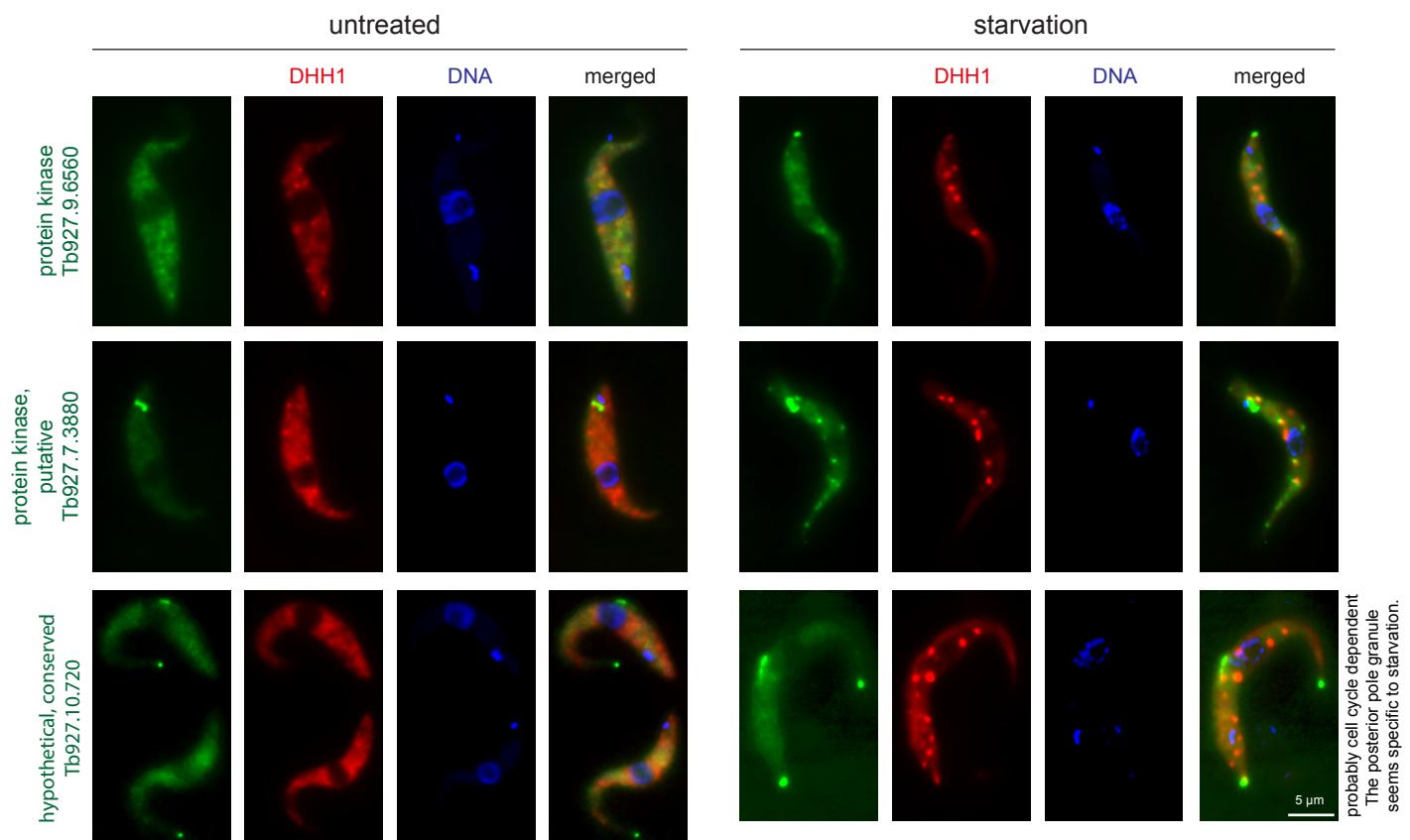


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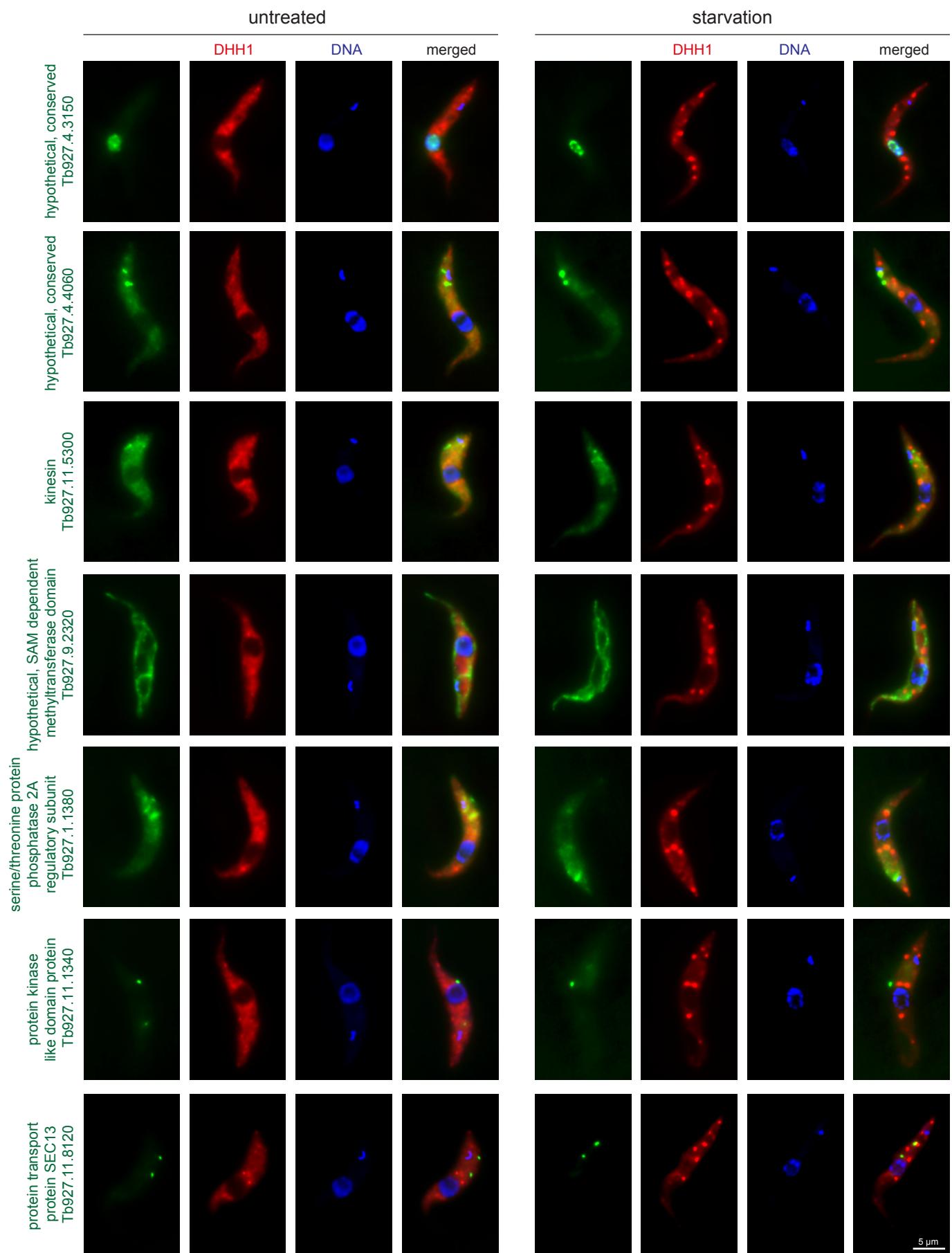


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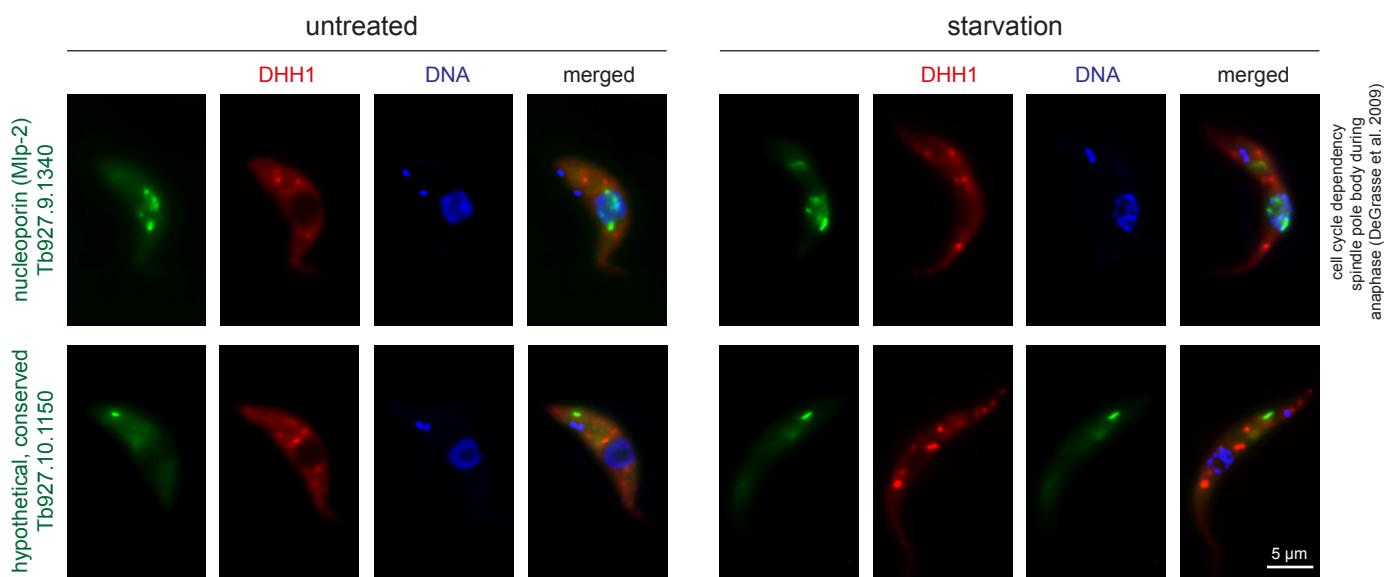


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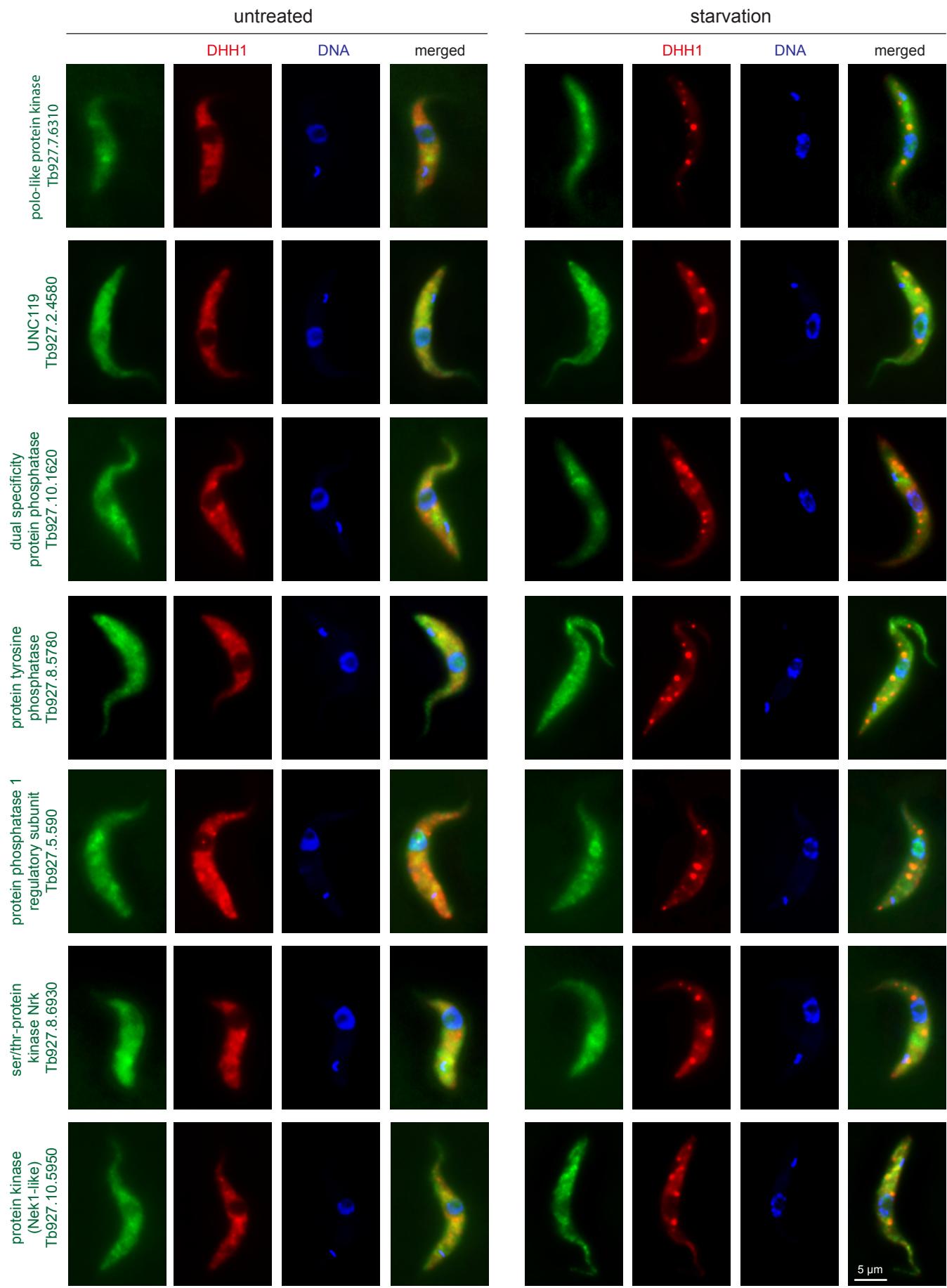


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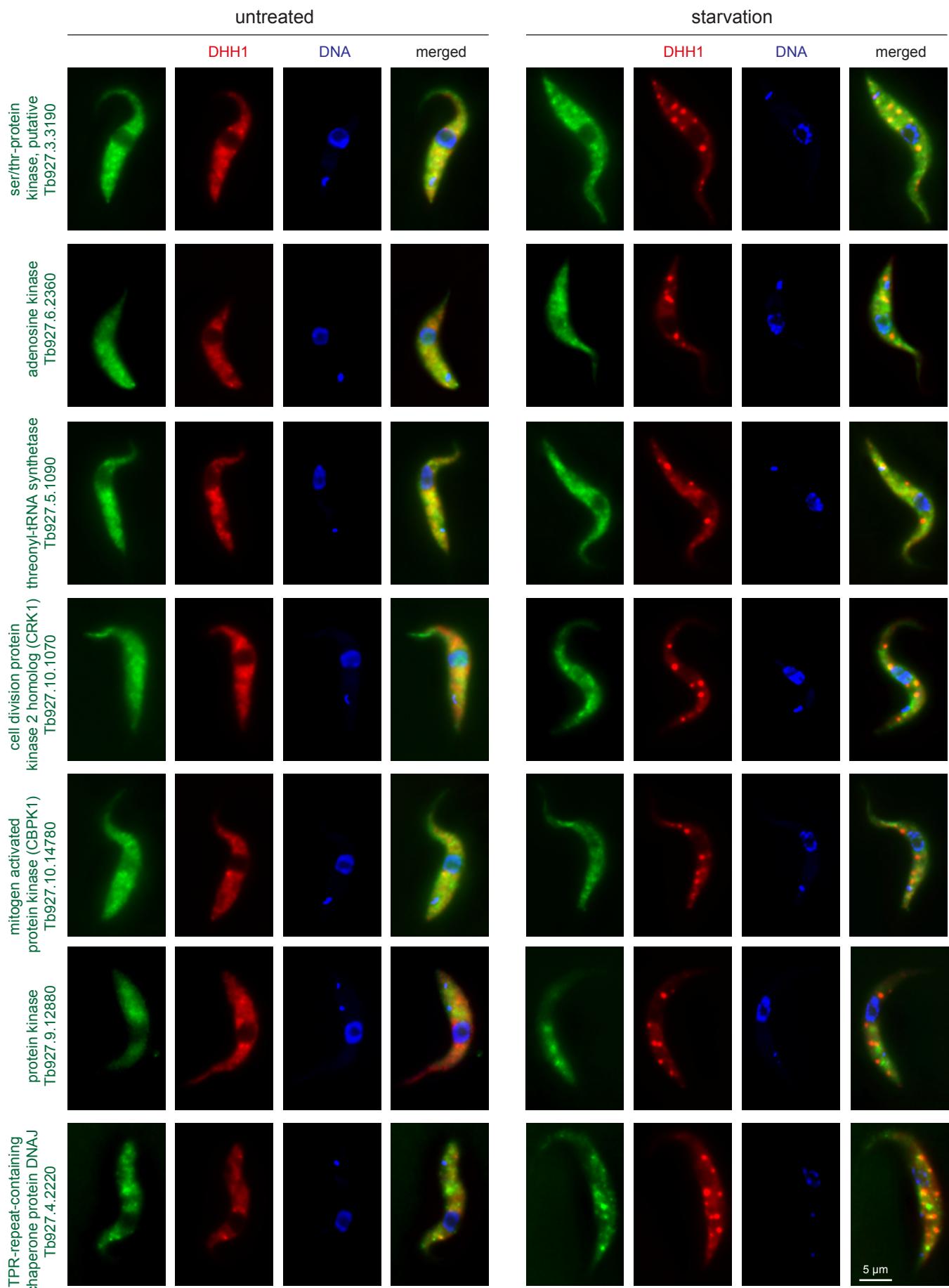


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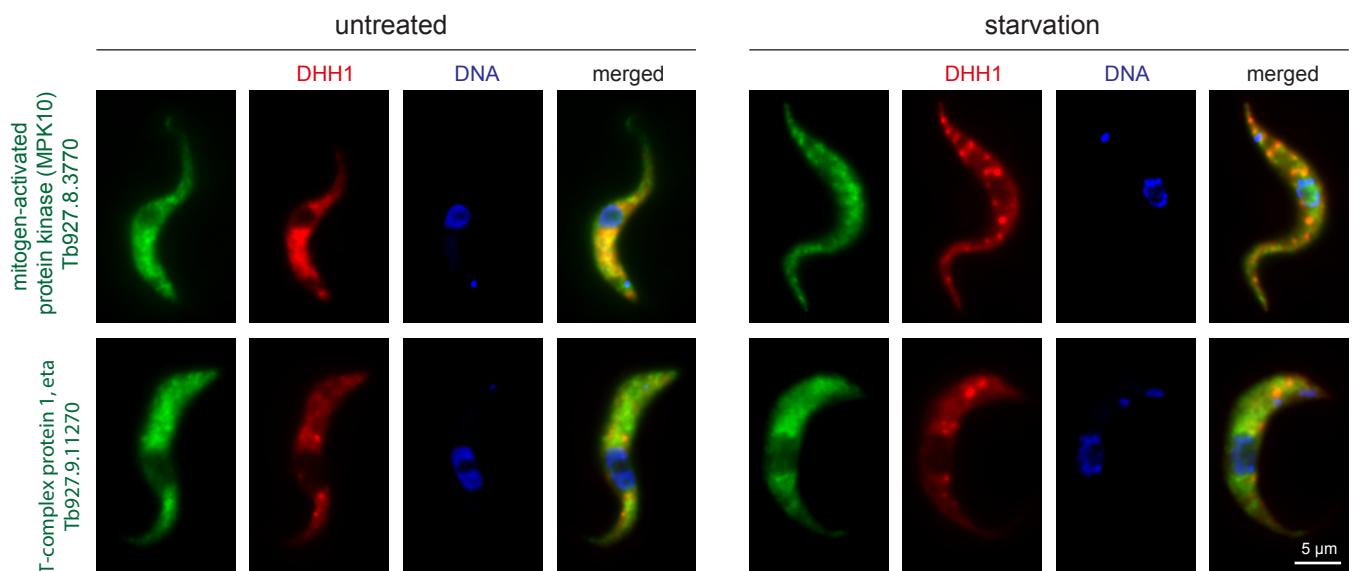


Figure S4. Validation of the mass spectrometry data by testing the localization of fluorescent protein fusions at starvation: proteins not involved in mRNA metabolism that do not localize to starvation stress granules

Fluorescence microscopy images of untreated and starved cells co-expressing the granule marker mChFP-DHH1 and the eYFP fusion of the granule candidate protein.

A) Proteins with a change in localization at starvation.

B) Proteins with a localization others than even cytoplasmic distribution.

C) Proteins with mainly cytoplasmic (and perhaps nuclear) distribution.

Figure S5

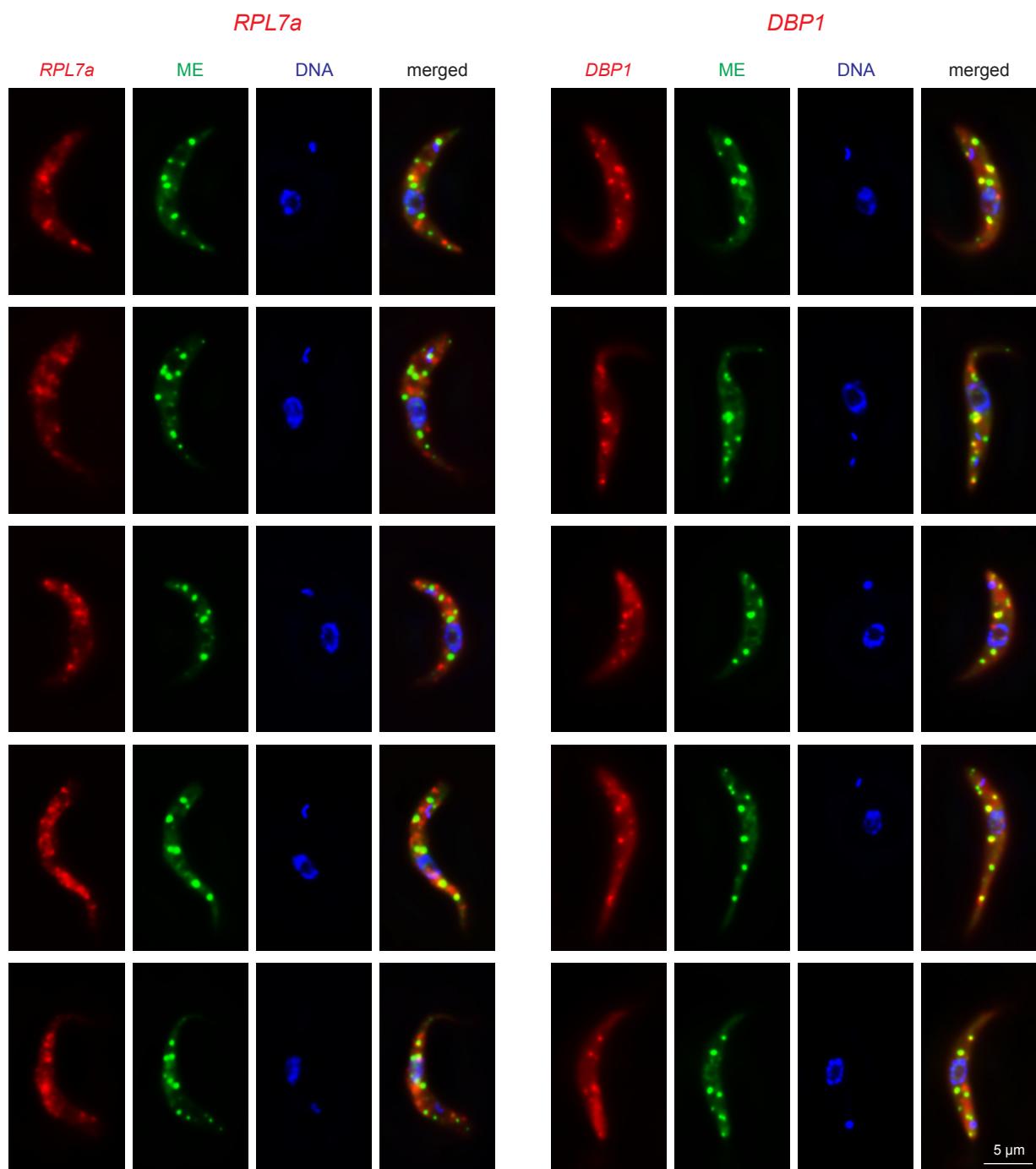


Figure S5. mRNA FISH of *RPL7a* and *DBP1* using the Stellaris system.
More images of starved cells are shown as sum slices of a deconvolved
Z-stack.

Figure S6

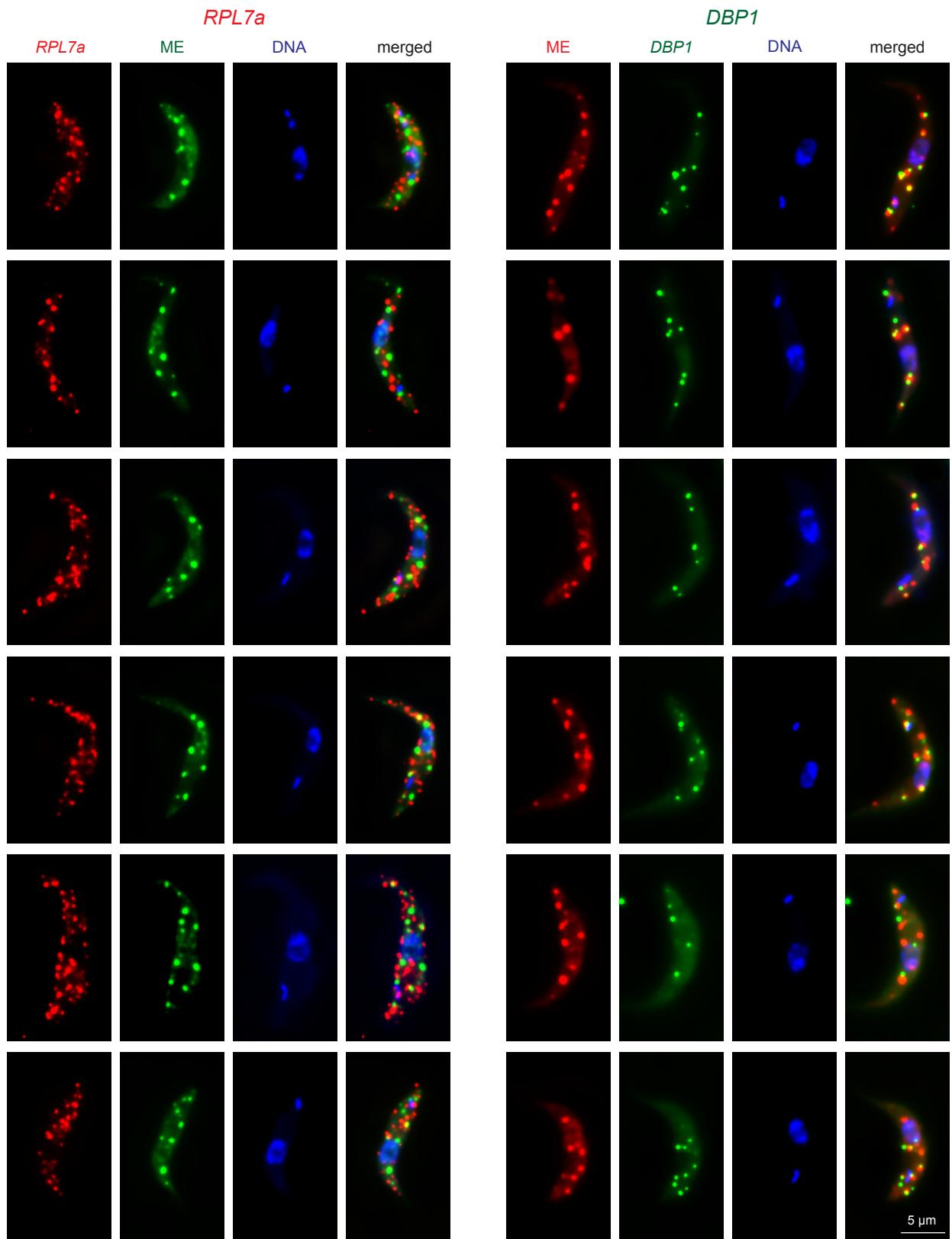


Figure S7

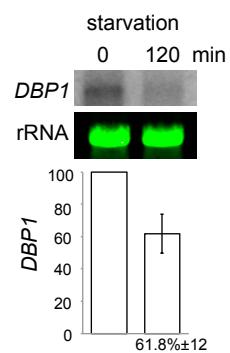


Figure S7

Northern blots loaded with total RNA of untreated and starved cells were probed for *DBP1* and rRNA (loading). The reduction in *DBP1* mRNA upon starvation was quantified from three independent experiments, of which one gel is shown.